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No effect of nutritional adenosine receptor antagonists on exercise performance in the heat

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Cheuvront SN, Ely BR, Kenefick RW, Michniak-Kohn BB, Rood JC, Sawka MN. No effect of nutritional adenosine receptor antagonists on exercise performance in the heat. *Am J Physiol Regul Integr Comp Physiol* 296: R394–R401, 2009. First published November 19, 2008; doi:10.1152/ajpregu.90812.2008.—Nutritional adenosine receptor antagonists can enhance endurance exercise performance in temperate environments, but their efficacy during heat stress is not well understood. This double-blinded, placebo-controlled study compared the effects of an acute dose of caffeine or quercetin on endurance exercise performance during compensable heat stress (40°C, 20–30% rh). On each of three occasions, 10 healthy men each performed 30-min of cycle ergometry at 50% $\dot{V}o_{2peak}$ followed by a 15-min performance time trial after receiving either placebo (*Group P*), caffeine (*Group C*; 9 mg/kg), or quercetin (*Group Q*; 2,000 mg). Serial blood samples, physiological (heart rate, rectal, and mean skin body temperatures), perceptual (ratings of perceived exertion, pain, thermal comfort, motivation), and exercise performance measures (total work and pacing strategy) were made. Supplementation with caffeine and quercetin increased preexercise blood concentrations of caffeine (55.62 ± 4.77 μM) and quercetin (4.76 ± 2.56 μM) above their in vitro inhibition constants for adenosine receptors. No treatment effects were observed for any physiological or perceptual measures, with the exception of elevated rectal body temperatures (0.20–0.30°C; $P < 0.05$) for *Group C* vs. *Groups Q* and *P*. Supplementation did not affect total work performed (*Groups P*: 153.5 ± 28.3, *C*: 157.3 ± 28.9, and *Q*: 151.1 ± 31.6 kJ; $P > 0.05$) or the self-selected pacing strategy employed. These findings indicate that the nutritional adenosine receptor antagonists caffeine and quercetin do not enhance endurance exercise performance during compensable heat stress.

caffeine; quercetin; thermoregulation; central fatigue; dietary supplements

ENDURANCE EXERCISE PERFORMANCE is impaired as ambient temperature increases (20, 26, 49). Although there are multifactorial physiological mechanisms that act to impair exercise performance in the heat (44), increased physiological strain is linked to a diminution in one or more central nervous system (CNS) functions. A disturbance in the balance of neurotransmitters has been offered as one categorical CNS factor responsible for early exercise fatigue (3), particularly when under heat stress (39, 46). Some support for this hypothesis includes improved endurance performance in hot, but not temperate, conditions after administration of a dopamine reuptake inhibitor in humans (52) and improved thermoregulation in rats after

intraperitoneal injection of a low-dose adenosine receptor antagonist (19). Because adenosine receptor antagonism can increase dopamine release (24) and may reduce heat strain in rats (19), the adenosine receptor appears a logical target when trying to nutritionally enhance endurance exercise performance in the heat.

Caffeine (1,3,7-trimethylxanthine) is a nutritional adenosine receptor antagonist (24) with proven efficacy for enhancing endurance exercise performance in temperate environments when consumed by humans over a wide range of doses (17, 28). The precise mechanism(s) behind caffeine's ergogenic quality remains unclear; but at relevant human doses (24), the findings of Davis et al. (14) provide evidence for adenosine receptor antagonism. Given caffeine's well-documented ergogenic effects over a broad range of different aerobic exercise tests (17), possible mechanism of action (14, 24) and the potential benefits of adenosine receptor antagonism in the heat (19, 52), it is surprising how few studies have investigated this potential. Two field studies with good ecological validity showed no benefits of caffeine on endurance time-trial performance when tested in hot, humid conditions (10, 21). However, changes in weather within trials, the inability to precisely match weather between trials, and the presence of substantial dehydration (3 to 4% of body mass) in both studies (10, 21) could have profoundly affected performance outcomes (44). Still another possibility is that secondary consequences of high heat stress, such as cardiovascular strain and more rapid glycogen depletion (44), might negate the performance benefits conferred by caffeine in more temperate environments (17). Although multiple stressors (heat, hydration, substrate availability) represent real-life challenges to athletes, soldiers, and others, they also add considerably to performance variability (8).

The flavanoid quercetin (3,3,4,5,7-pentahydroxyflavone) (1, 34) is another nutritional adenosine receptor antagonist that has received media and military attention as a possible ergogenic aid (35). The in vitro affinity of unconjugated quercetin for adenosine receptor subtypes appears similar (1) or greater (34) than caffeine (24); therefore, quercetin could mimic or surpass caffeine's effects on endurance exercise performance if adenosine receptor antagonism is the mechanism. Although the in vivo activity of conjugated quercetin may be lessened (16), adenosine receptor antagonism remains a tenable hypothesis (16, 34). While the only study to examine the potential for quercetin to enhance endurance exercise performance (35)

reported small, but significant, improvements in a 30-km cycle time trial in a temperate environment, the interpretation of findings is complicated by the use of a delivery vehicle containing several other bioactive ingredients. Unlike caffeine, quercetin also has numerous potential health benefits that include the possibility of reducing viral illness and antioxidant and anti-inflammatory properties (11, 15, 31, 37). This makes quercetin an attractive alternative to caffeine, provided that it confers similar exercise performance benefits. The isolated effects of quercetin on endurance exercise performance have not yet been examined in any environment.

The purpose of this study was to determine the independent effects of acute caffeine or acute quercetin ingestion on endurance exercise performance in the heat. Our hypothesis was that both caffeine and quercetin would improve endurance exercise performance. Dehydration was carefully prevented and the exercise task (preloaded time trial) and duration (45-min) were selected with the purpose of testing the aerobic energy system while minimizing any influence of energy depletion on performance. In addition, a hot, but compensable, testing environment was selected that would not limit performance due to excessive hyperthermia (38, 44).

MATERIALS AND METHODS

Subjects. Ten healthy, non-heat-acclimated male volunteers [mean (range): age 23 (18–37) yr, body mass 77.5 (64.2–94.8) kg, height 178 (169–188) cm, body fat 12 (7–19) %] participated in this study and completed all phases of experimentation. Subjects were physically active and moderately fit [mean (range); peak oxygen uptake ($\dot{V}_{O_{2peak}}$) of 45.2 (40.5–55.2) ml·kg^{-1}·min^{-1}]. A caffeine intake questionnaire was given, and anyone admittedly sensitive to caffeine or who consumed > 400 mg/d was excluded from participation. Caffeine abstinence was required for 4 days before testing (22). Volunteers were also asked to refrain from physical activity and alcohol consumption 24 h before testing. The U.S. Army Research Institute of Environmental Medicine Human Use Review Committee approved this study. Subjects were provided informational briefings and gave voluntary, informed written consent to participate. Investigators adhered to AR 70–25 and U.S. Army Medical Research and Materiel Command Regulation 70–25 on the use of volunteers in research.

Preliminary procedures. Two weeks of preliminary testing were completed in a temperate environment (20–22°C) prior to the experimental trials. $\dot{V}_{O_{2peak}}$ was measured in all volunteers using an incremental cycle ergometer protocol with continuous gas-exchange measurements (True-Max, ParvoMedics, Sandy, UT). The ergometer used for all exercise testing (Lode Excalibur Sport, Lode, Groningen, The Netherlands) allows pedal rate-independent (hyperbolic) and -dependent (linear) modes of cycling. Linear factors and pedal cadences were determined as previously described for a similar test population (8).

Volunteers performed an average of three familiarization trials, including one while fully instrumented, to reduce training and learning effects while also establishing a reliable best time-trial performance (27, 32). Familiarization trials began with 30-min of steady-state cycle ergometry at 50% of $\dot{V}_{O_{2peak}}$ (hyperbolic mode). This was followed by a brief 5-min rest period, after which a 15-min time trial (linear mode) was completed. These sessions mimicked experimental trials in every way except for dietary supplement intervention and the temperate environmental conditions (20–22°C). During the time trial, volunteers were blinded to all but elapsed time and no motivation was given. Once completed, volunteers were provided with feedback on their performance, defined as the total amount work (kJ) completed in 15 min, as motivation to improve with each subsequent training bout.

Volunteers were also familiarized with all perceptual scales at this time. The choice of exercise task was selected: *1*) to reduce the potential influence of the aforementioned confounders related to dehydration and excessive heat stress, *2*) because it is a reliable test consistent with recommendations for studying the ergogenic potential of food components (29, 32, 42), and *3*) it would allow a reasonable comparison to performance in the Army 2-mile run, which is similar in duration and places demands on the same energy system. The choice of test is also consistent with previous studies of caffeine ergogenics (17).

During preliminary testing, volunteers reported each morning after an overnight ~8-h fast for first-void urine-specific gravity (USG) and nude body mass measurements. To ensure proper hydration, 30 ml/kg of fluid electrolyte beverage was provided supplemental to ad libitum fluid intakes and was directed for consumption the evening before each training day. An average of the 5–10 days of nude body mass measurements were calculated and used as a baseline reference for later euhydrated body mass determinations.

Experimental procedures. Volunteers were randomly assigned to complete three experimental trials [placebo (*Group P*), caffeine (*Group C*), quercetin (*Group Q*)] separated by 5–7 days each. All experiments were conducted at the same time of day to control for circadian fluctuations in body temperature and other biological variables (48). The elapsed time between trials was also considered adequate to prevent heat acclimation (4). Volunteers drank 30 ml/kg of fluid electrolyte beverage the night before each test and arrived the next morning after an overnight fast for nude body mass and first-void USG analysis. Volunteers with a combination of any two USG < 1.02, nude body mass within 1% of the 2-wk average, or plasma osmolality < 290 mOsmol/kg H_2O were considered euhydrated (45). A fasting blood sample was drawn (baseline), after which volunteers were given a standardized breakfast including sports bars, gelatin capsules, and water. A second blood sample was drawn 1-h post-breakfast (preexercise). Volunteers were then instrumented in the 20–22°C antechamber (~10 min) and weighed immediately upon entering the hot test environment (40°C, 20–30% rh).

Following a seated 20-min stabilization period in the 40°C test environment, data collection was begun. Thirty minutes of steady-state cycle ergometry was completed at 50% $\dot{V}_{O_{2peak}}$ intensity. The combination of environmental and exercise heat loads was compensable ($E_{req} < E_{max}$) for this phase of the study and calculated to keep rectal temperature (T_{re}) below our laboratory safety cutoff of 39.5°C. This minimized the potential for having to stop the ensuing time trial as a result of reaching T_{re} safety limits. Drinking was not permitted during exercise, but a 5-min break followed wherein subjects were weighed and rehydrated to within 1% of the instrumented body mass measured previously upon chamber entry. Volunteers then completed a 15-min performance time trial as previously described for familiarization sessions, except that no performance feedback was given. A third blood sample was drawn immediately upon time trial completion (postexercise). Performance was assessed as the total work (kJ) completed in 15 min. Percentage changes in performance (e.g., relative to placebo) were calculated so that positive and negative values reflect more and less work, respectively. In an effort to more fully understand performance, the potential for supplementation to affect pacing (2) was also examined. Briefly, individual volunteer pacing was evaluated by comparing the difference in actual work performed in each of five 3-min work blocks normalized to the arithmetic mean (15/5). A negative number indicates a slower-than-average pace for that block; a positive number, a faster than average pace.

Body mass was measured nude with an electronic precision balance scale (accuracy ± 50 g; model WSI-600; Mettler, Toledo, Columbus, OH) before and after breakfast, as well as after the exercise session. Fully-instrumented body mass was measured in the chamber before and after steady-state exercise to determine fluid needs in the rest period preceding the time trial. Gas exchange measurements were made once after the initial 15 min of steady-state exercise using an

automated system (TrueMax, ParvoMedics, Sandy, UT). Heart rate (HR) (Polar a3; Polar Electro, Woodbury, NY) was recorded at 5-min intervals, as was T_{re} obtained from a telemetric temperature sensor (Jonah core body temperature capsule; Mini Mitter, Bend, OR) inserted 8–10 cm beyond the anal sphincter. Simultaneous pilot testing of this approach against a conventional rectal probe yielded excellent agreement ($\leq 0.05°C$ difference; $n = 3$), and the techniques were considered equivalent. Skin temperature (T_{sk}) was monitored continuously at the left chest, arm, calf, and thigh using thermistors (YSI, Yellow Springs, OH) linked to a data acquisition system. Mean weighted T_{sk} was calculated according to Ramanathan (40). Ratings of perceived exertion (RPE) and pain (RP) (7) were measured serially using the appropriate numerical scales anchored by verbal descriptors. Ratings of motivation (RM) (30) and thermal comfort (RTC) (25) were also determined using measured distance (mm) on a continuum scale between verbal anchors.

Breakfast each day consisted of four cranberry-flavored energy bars (total energy = 557 kcal; 78% CHO, 18% fat, 4% protein), similar to commercially available sports bars. Other ingredients (sodium, potassium, chloride, calcium, vitamin C, and vitamin E) were well below ordinary U.S. Dietary Reference Intake levels. Volunteers also consumed between four and seven gelatin-coated capsules with ~500 ml water. The energy bars (Combat Feeding Directorate, U.S. Army Natick Soldier RDEC) were the delivery vehicle for food-grade quercetin aglycone powder (QU995; Quercegen Pharma, Newton, MA) equal to 500 mg/bar (2,000 mg total). The placebo bars contained no quercetin. The capsules delivered either USP anhydrous caffeine or microcrystalline cellulose (placebo) at 9 mg/kg. Capsules were formulated (Compounded Solutions in Pharmacy, Monroe, CT) to contain from 5 to 200 mg each to allow precision (± 0.1 mg/kg) dosing. Volunteers received the placebo capsules and placebo bars (*Group P*), caffeine capsules and placebo bars (*Group C*), or placebo capsules and quercetin bars (*Group Q*). Treatment and placebo bars and capsules were indistinguishable by flavor and by ordinary inspection. Treatments were double blinded, and trial order was determined using a Latin square assignment. The doses for both caffeine and quercetin were estimated to elicit blood concentrations in excess of the in vitro inhibition constant (K_i) for adenosine receptor antagonism (caffeine, ~25 μM; quercetin, ~2.5 μM) (24, 34). The chosen dose for caffeine was also consistent with enhancing endurance exercise performance (2, 17, 28).

Blood and urine analysis. Venous blood samples were collected from a superficial antecubital vein. Baseline 10-ml samples were drawn in the fasted state (~8 h) after volunteers had been quietly seated for a 15-min stabilization period with arm position standardized. This sample was drawn immediately after the first morning weight and urinalysis. The second 10-ml sample was drawn 1 h after the breakfast and supplements were consumed, with subjects again seated quietly for 15-min before sample collection. Both preexercise samples were drawn in the antechamber environment. The third 10-ml sample was drawn in the test environment immediately upon time trial completion with the volunteers still seated on the cycle ergometer.

Blood from heparinized tubes was centrifuged for 10 min at 4°C, and plasma aliquoted for the measurement of glucose and lactate (YSI 2300 STAT). Plasma from EDTA tubes was aliquoted and analyzed for plasma osmolality by freezing point depression (model 210 Micro-Osmometer; Fiske, Norwood, MA) or treated and stored frozen (−80°C) for glycerol and quercetin analyses. Glycerol, which was selected as a minimally confounded estimate of lipolytic activity, was measured using standard enzymatic fluorometric methods (Boehringer-Mannheim Kit 148270). Quercetin was measured from plasma that was immediately centrifuged for 15 min at room temperature, after which 100 μl of 10% ascorbic acid (Sigma Aldrich, St. Louis, MO) was added. The mixture was vortexed for 30 s and immediately frozen until analysis. The assay of quercetin present in plasma samples was performed by solid-phase extraction technique after converting quercetin metabolites to unconjugated quercetin with enzyme β-glucuron-

idase/sulfatase. The quercetin analysis was performed using HPLC integrated with a UV detector (HP1100 with Agilent Chemstation software). Plasma caffeine was determined from blood collected in a no-additive tube and held on ice for 30-min prior to centrifugation and freezing. Analysis was performed using a Beckman Coulter DXC 600 Pro and EMIT reagents for caffeine (Dade-Behring Diagnostics, Deerfield, IL). The assay is based on competition for antibody binding sites between caffeine in the sample, and caffeine labeled with the enzyme glucose-6-phosphate dehydrogenase. USG was measured by refractometry (1110400A TS Meter; AO Reichert Scientific Instruments).

Statistics. The effects of treatment (caffeine, quercetin, placebo) on outcome variables of interest was assessed using a one-way (trial) or two-way (trial $\times$ time) repeated-measures ANOVA. Where the assumption of sphericity was violated, *F* values were adjusted using Greenhouse-Geisser or Huynh-Feldt corrections as appropriate. Tukey's honestly significant difference procedure was used to identify differences among means following significant main and/or interaction effects. Where indicated, the error uniformity of some variables was tested using regression analysis. The primary outcome variable of interest in this experiment was time-trial performance. A minimum sample size of 6–7 (50) was calculated ($\alpha = 0.05$, $\beta = 0.20$) as sufficient to detect a 5% change (~10 kJ) in time-trial performance from placebo in the ANOVA. This number was estimated using the best performances (186 $\pm$ 32 kJ) achieved during the initial 2-wk of performance familiarization training ($n = 9$) and from the within-subjects coefficient of variation expressed as a percentage of the mean (4.5%) for trials of no difference (Grubbs' test). An effect size of >1.0 was selected based on the likelihood of experimental perturbations producing unique performance infidelity (32), which would decrease the observed signal-to-noise ratio. A sample size of 10 volunteers was tested to allow detection of desired differences with an effect size as small as 0.7 (50), to guard against possible attrition, and to afford more confidence that our volunteers were representative of their wider population. Graphical data are presented with unidirectional error bars for presentation clarity. All data are presented as means $\pm$ SD except where indicated.

RESULTS

Hydration. Hydration status was assessed on the morning of each trial. There were no significant differences ($P > 0.05$) among trials for first morning measures of body mass, USG, or plasma osmolality. The means and standard deviations for *Groups P, C,* and *Q* were body mass (%) (0.58 $\pm$ 1; 0.63 $\pm$ 1; 0.59 $\pm$ 1), USG (1.016 $\pm$ 0.006; 1.014 $\pm$ 0.006; 1.017 $\pm$ 0.006), and plasma osmolality (mOsmol/kg H_2O) (289 $\pm$ 4; 290 $\pm$ 4; 288 $\pm$ 4), respectively. Means include individual volunteers who exceeded euhydration thresholds at the first morning measurement (6/30 for USG; 4/30 for plasma osmolality) before being given a mandatory 500 ml of water to drink with breakfast. Volunteers were therefore considered equally and normally euhydrated at the start of each trial (45). The level of dehydration incurred during 30 min of steady-state cycle ergometry was also not different among trials ($P > 0.05$), averaging a modest 0.7 $\pm$ 0.1%. Volunteers were then given an average of 240 $\pm$ 7 ml of water to replace the losses.

Physiological responses. Metabolic rates during 30-min steady-state exercise were calculated from the average of a 3-min gas sample made 15-min into exercise. Intensity was expressed as a percentage of $\dot{V}_{O_{2peak}}$ and was similar among all trials ($P > 0.05$) at 51.2 $\pm$ 2.0% (*Group P*), 52.5 $\pm$ 2.1% (*Group C*), and 51.8 $\pm$ 3.1% (*Group Q*); thus exercise was properly matched for intensity among treatments preceding the time trial. Figure 1, *A–C* represents HR, T_{re}, and T_{sk} responses
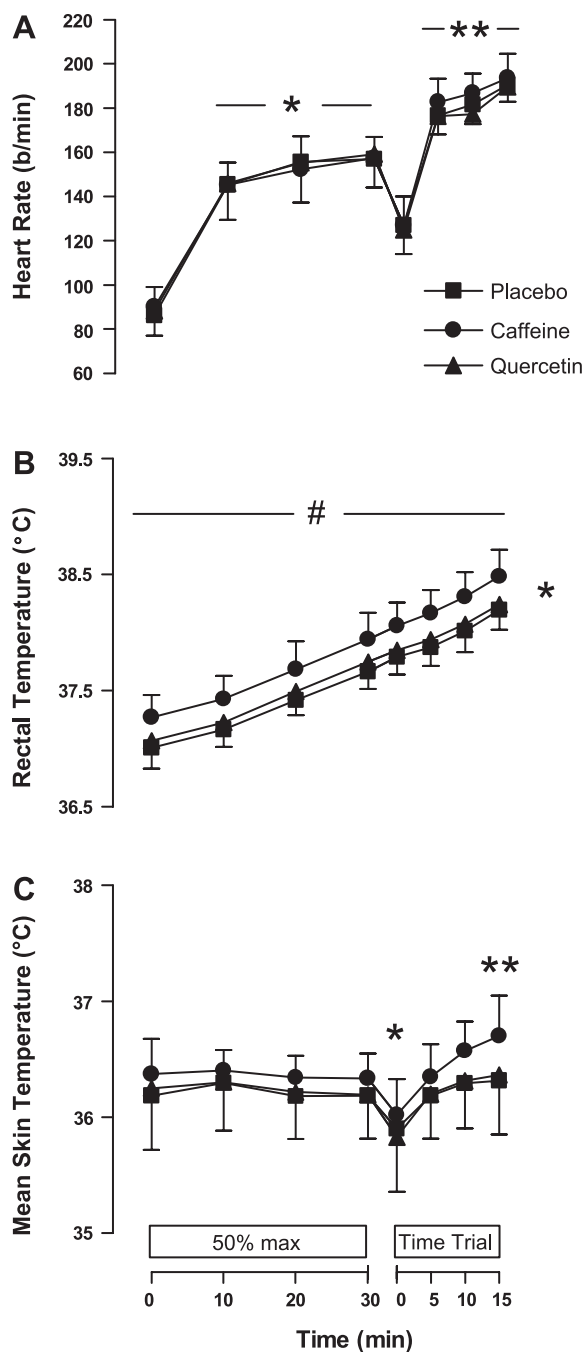
Fig. 1. Effects of supplementation with caffeine or quercetin on heart rate (*A*), rectal temperature (*B*), and mean skin temperature (*C*). *A*: main effect of time; *10–30 min > 0 min (steady-state) and 0 min (time trial), **5–15 min > all. *B*: main effect of trial, #Group C > Group Q and Group P; main effect of time, *progressive differences at all time points. *C*: main effect of time, *0 min (time trial) < all, **15 min (time trial) > 20 and 30 min (steady-state) and 0, 5 min (time trial). All notations are significantly different (*P* < 0.05).

to exercise across time. HR increased (*P* < 0.05) over time during exercise and had the anticipated additional increase during the time trial (Fig. 1*A*). At the completion of the 15-min time trial, HRs were at 98.3 ± 5.6% (*Group P*), 98.2 ± 4.9% (*Group C*), and 97.1 ± 5.2% (*Group Q*) of the age-predicted maximum. T_{re} increased (*P* < 0.05) over time during exercise and was significantly elevated (0.20–0.30°C; main effect of

trial) throughout *Group C* compared with *Groups P* and *Q* (Fig. 1*B*), but the change in T_{re} was not different among *Groups P* (1.19 ± 0.24°C), *C* (1.21 ± 0.18°C), or *Q* (1.17 ± 0.25°C) (*P* > 0.05). T_{sk} remained relatively constant during exercise and appeared to increase more in *Group C* during the time trial, but only a main effect of time was observed (Fig. 1*C*). Sweating rates, calculated from the change in body mass after 30-min of steady-state exercise, were not different among trials (*Group C* = 0.70 ± 0.14 l/h; *Group P* = 0.66 ± 0.11 l/h; *Group Q* = 0.67 ± 0.12 l/h) (*P* > 0.05).

Blood. Table 1 provides the blood concentration values among trials. The blood levels of caffeine in *Groups P* and *Q*, and quercetin in *Groups C* and *P*, were near or below trace amounts for each. This indicates compliance with dietary caffeine restrictions and confirms the low level of quercetin in the ordinary Western diet, and possibly seasonal (winter) food availability (11). There were no side effects noted (withdrawal or dose-related) for caffeine or quercetin. Caffeine supplementation at 9 mg/kg (9.00 ± 0.03 mg/kg, average dose 694 ± 85 mg) elevated blood concentrations in excess (Table 1) of caffeine's K_i for the adenosine receptor (~ 25 µM) (24) within 1 h after ingestion. This was true also for the 2,000 mg dose of quercetin (Table 1), which raised blood concentrations above the flavanoid's K_i of 2.5 µM (34). All blood variables tended to increase in response to exercise, and subtle differences were noticed for glucose and glycerol between *Groups C* and *Q*. In addition, lactate was significantly (*P* < 0.05) elevated immediately after exercise in *Group C* compared with *Groups Q* and *P* (Table 1), similar to what others have observed for caffeine doses > 3 mg/kg (e.g., see Ref. 28).

Perceptual responses. Table 2 provides perceptual scale results for all trials. There were no differences observed among trials on RPE, RP, RTC, or RM. As expected there were higher RPE and RP reported at the completion of the time trial than during steady state in all trials. RTC was also reported higher at the completion of the time trial vs. steady state for *Groups C* and *Q*, but not *Group P*. No effects of time were reported for RM (Table 2).

Exercise performance. Table 3 provides individual and mean 15-min time-trial performance data. Neither supplementation with caffeine nor quercetin had any impact on the total work performed. Relative to *Group P*, the mean percentage change in performance for *Group C* (3%; 95% CI = −4.9 to 11.0%) and *Group Q* (−1.2%; 95% CI = −9.4 to 7.0%) was small but highly variable. The uniformity of the %change in performance from *Group P* was examined by inspection of the residuals from the regression analysis of %change in performance (*y*-axis) against kJ of work completed (*x*-axis). This analysis indicated greater individual response variability for volunteers who completed < 150 kJ of work in response to caffeine or quercetin supplementation (11 of 20 trials). The results for trial order, independent of treatment, were also similar for *Trial 1* (155.1 ± 23.4 kJ), *Trial 2* (155.0 ± 33.9 kJ), and *Trial 3* (151.7 ± 31.8 kJ) (*P* > 0.05). Figure 2 shows that pacing was also unaffected by supplementation. In all trials, pacing across the five 3-min blocks of work indicated a tendency to begin and end the time trial faster than the normalized average (above zero). Statistically, pacing dropped off in blocks 3 and 4 (< block 1) and remained lower in block 4 compared with block 5 (*P* < 0.05) (Fig. 2). Figure 3 compares the baseline peak total work achieved during training

Table 1. *Comparison of select blood concentrations among trials*

Measure	Placebo			Caffeine			Quercetin		
	Baseline	Pre-Ex	Post-Ex	Baseline	Pre-Ex	Post-Ex	Baseline	Pre-Ex	Post-Ex
Glucose, mM	5.30±0.53	5.45±1.31	6.38±0.79*†	5.45±0.63	5.27±0.90	6.95±0.97*†	5.49±0.35	5.41±1.37	6.15±1.05[a]
Lactate, mM	1.17±0.43	2.47±0.81	8.46±2.41*†[a]	1.43±0.39	2.90±0.69	11.67±3.79*†	1.27±0.35	2.44±0.47	8.28±3.93*†[a]
Glycerol, mM	0.06±0.02	0.04±0.02	0.19±0.05*†	0.05±0.03	0.06±0.04	0.22±0.07*†	0.05±0.03	0.06±0.03	0.17±0.05*†[a]
Caffeine, µM	0.26±0.36	0.21±0.36[a]	0.05±0.16[a]	0.31±0.65	55.62±4.77*	71.22±16.58*†	1.54±2.02	1.13±1.92[a]	0.72±1.14[a]
Quercetin, µM	0.02±0.00	0.02±0.00[b]	0.02±0.00[b]	0.02±0.00	0.02±0.00[b]	0.02±0.00[b]	0.02±0.00	4.76±2.56*	7.68±3.77*†

Values are means ± SD. Different from baseline (*) or Pre-Ex (†) within trials; Different from caffeine (a) or quercetin (b) among trials at the same corresponding time points ($P < 0.05$).

in temperate conditions (20–22°C) with the three experimental trials in the heat. Performances in the heat were significantly reduced ($P < 0.05$) by −18.7 ± 9.2% (*Group P*), −16.4 ± 6.5% (*Group C*), and −18.3 ± 9.2% (*Group Q*), respectively.

DISCUSSION

This is the first study with laboratory control to evaluate the impact of acute nutritional adenosine antagonist (caffeine and quercetin) administration on endurance exercise performance in the heat. An underlying assumption was that caffeine and quercetin might improve performance by adenosine receptor antagonism (14). Indeed, the actions of caffeine and quercetin on adenosine receptors is well described (1, 24, 34) and the blood concentrations achieved in this study were in excess of the in vitro adenosine receptor K_i (24, 34). The dose selected for caffeine was also consistent with exercise performance enhancement in time to exhaustion (28) and time-trials tests (2) in temperate environments and for aerobic exercise in temperate environments in general (17). Control over time of day, hydration, activity, and antecedent diet (morning meal and caffeine restriction) was employed, and the exercise task and duration were selected with the purpose of maximizing performance repeatability while minimizing the potential influences of energy depletion and excessive heat stress. The principle findings of this study were: *1*) acute supplementation with caffeine or quercetin did not improve endurance exercise performance in the heat, and *2*) acute supplementation with caffeine, but not quercetin, had modest thermogenic effects during exercise in the heat.

A possible reason for the null findings reported herein may be that adenosine receptor antagonism is not responsible for the ergogenic effects of caffeine. Evidence for a CNS adenosine antagonism mechanism was proposed (14) based on the following observations in temperate conditions: *1*) impaired exercise performance in rats administered an adenosine drug agonist, *2*) the reversal of this effect with caffeine, and *3*) improved run time to exhaustion with caffeine only. Evidence points 2 and 3 were both achieved with a small (0.6 mg/kg) intracerebroventricular dose of caffeine, while the same intraperitoneal dose had no effects. It remains possible that much larger intraperitoneal doses, incompatible with human ingestion (> 20 mg/kg) (24), might be required to elicit similar drug reversal effects (reviewed in Ref. 14). Therefore, the fact that comparably smaller oral doses of caffeine can improve endurance exercise performance in both rats and humans in temperate conditions raises questions about the precise roles of both the CNS and heat stress in this process. Similar questions abound for the less-studied quercetin, including uncertainty about conjugated quercetin's affinity for adenosine receptors in vivo (16, 34), optimal dosing protocols, and precise mechanism(s) of action.

The thermogenic potential of caffeine to increase physiological strain in a hot environment has not generally been observed (6, 12, 41, 47), making it an unlikely explanation for null performance findings. The combination of high environmental heat stress and large dose of caffeine (9 mg/kg) in this study elevated T_{re} in *Group C* (0.20–0.30°C; $P < 0.05$) only modestly (Fig. 1*B*). This effect of caffeine was consistent for all volunteers and has been reported before under similar conditions (47). Higher exercise lactates in *Group C* (Table 1) favor a higher metabolism to explain this observation, but no differences in steady-state exercise HR or $\dot{V}_{O_2}$ argue against this hypothesis. Although the mechanism remains unclear from the measurements made, the change in T_{re} was not different among trials and the subtle but constant higher body temperatures in *Group C* were not enough to produce differences in gross measures of whole body sweating. Similarly, although RTC was higher at the completion of the time trial compared with steady-state exercise in *Group C*, it was also higher in *Group Q* where T_{re} was not different from *Group P*. Importantly, no differences in RTC were observed relative to *Group P* for *Groups C* or *Q* (Table 2).

Although exercise duration and hydration status were controlled, the percentage of HR_{max}, T_{re}, T_{sk}, and perceptual

Table 2. *Perceptual performance data during final minute of steady-state (SS) and time trial (TT) exercise*

Measure	Placebo		Caffeine		Quercetin	
	SS	TT	SS	TT	SS	TT
RPE	10.9±2.4	17.4±2.9*	9.8±2.0	16.3±3.2*	11.4±2.1	17.0±1.8*
RP	1.0±1.2	3.7±3.3*	1.0±1.0	4.2±3.0*	1.5±1.6	3.6±3.1*
RTC, mm	57.0±20.0	69.0±22.5	50.4±21.6	72.8±15.5*	48.7±16.6	66.6±21.6*
RM, mm	89.9±28.9	93.8±21.2	93.1±23.1	90.5±18.2	82.3±24.2	80.2±34.9

Values are means ± SD. *Different from SS within trials ($P < 0.05$). RPE, rating of perceived exertion; RP, rating of pain; RTC, rating of thermal comfort; RM, rating of motivation.

Table 3. *Fifteen-minute time trial work performance (kJ)*

Subject No.	Placebo	Caffeine	Quercetin
1	123.6	144.3	138.2
2	155.8	157.2	144.2
3	140.8	115.3	114.8
4	113.7	118.4	116.0
5	180.4	175.2	143.9
6	182.1	181.2	195.0
7	196.7	211.9	213.3
8	129.4	157.7	141.7
9	172.8	165.7	163.6
10	139.3	145.8	140.7
Mean	**153.5**	**157.3**	**151.1**
SD	**28.3**	**28.9**	**31.6**

responses (Fig. 1, *A–C*, Table 2, and text) still reflect substantial physiological strain, and heat stress drastically reduced (>15%) performance relative to the temperate training environment (20–22°C). While this comparison is not perfect, as it was not the intent of this study to draw this contrast, it is insightful for gauging the impact of heat stress per se on performance. Tyler and Sunderland (51) also reported a −10% decrement in a 15-min treadmill time trial in warm (30°C) vs. cool (14°C) conditions following a longer 75-min preload. Watson et al. (52) likewise reported a −30% performance decline in the warmer conditions for nine men during a ~30-min time trial after a 60-min preload. While there is evidence that a "critical" core body temperature (≥40°C) can impair performance through reduced neural drive and muscle force production (38), performance was impaired in this study with core body temperatures of <39°C. Because exhaustion from heat strain can occur across a broad range of core body temperatures (43), it is plausible that other factors related to environmental heat stress overwhelm the mechanism(s) responsible for central (13) or peripheral nutritional ergogenics. If so, a similar phenomenon may explain the absence of ergogenic effects for carbohydrate supplementation in warm,

Fig. 3. Percentage decrease in time-trial performances in the heat (40°C) compared with best training values observed in a more temperate environment (20–22°C). P, placebo; C, caffeine; Q, quercetin. The value marked with an arrow was a statistical outlier (Grubbs' test) but was retained in the calculations presented in the text. Bars and whiskers represent means ± SD (n = 9). Shaded region represents the within-subjects coefficient of variation (4.5%) during training.

humid conditions (36), otherwise consistently observed in temperate environments. Exhaustion from heat strain, in this context, remains highly relevant for many situations (43).

The psychological impact of dietary supplements must also be considered. In a meta-analysis of caffeine's effects on perceived exertion, Doherty and Smith (18) suggested that the ~5% absolute reduction in RPE afforded by caffeine may account for ~30% of its ergogenic effects. Volunteers in this study practiced use of the perceptual scales prior to experimentation to maximize their sensitivity. The within-trial responses for pain and motivation were very similar for *Groups C, P,* and *Q* (Table 2). Although like others (17) we observed an absolute reduction in RPE (~5%) in *Group C* relative to *Groups Q* and *P*, this effect did not approach statistical significance. But creative studies (5, 23) investigating the placebo or nocebo effects of caffeine supplementation indicate that belief or disbelief can produce an independent effect of similar magnitude. Treatment uncertainty or expectation reduces measurement precision (9), which may contribute to the null findings reported after double-blinded study of well-recognized ergogenic supplements (e.g., carbohydrate). This is true even in large (n > 40) studies with trained athletes (9) and has been observed in both temperate (9) and warm environments (36).

If the mean performance outcomes reported in this study represent the true effect of the treatment for the population studied, then caffeine and quercetin have small-to-no effect on endurance exercise performance in the heat. This corroborates a small number of like studies (10, 21). The absence of a treatment effect on pacing is supporting evidence since some studies (2) have found that only the early phase (first 25%) of a performance task contributes to the total ergogenic effect of caffeine. In absolute terms, acute caffeine or quercetin ingestion improved performance only six times out of 10 (Table 3), a result (P > 0.05) explainable by chance. While it is true that
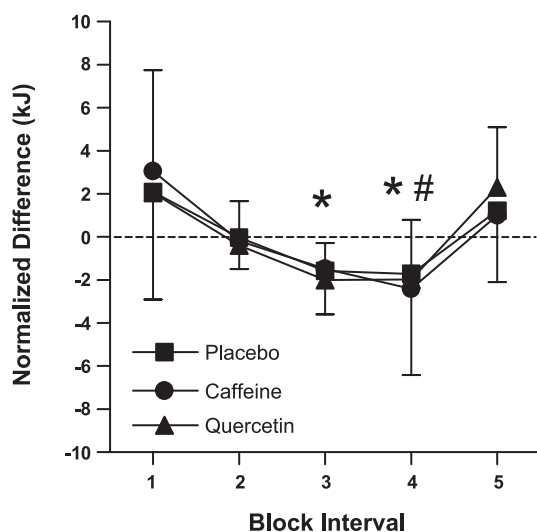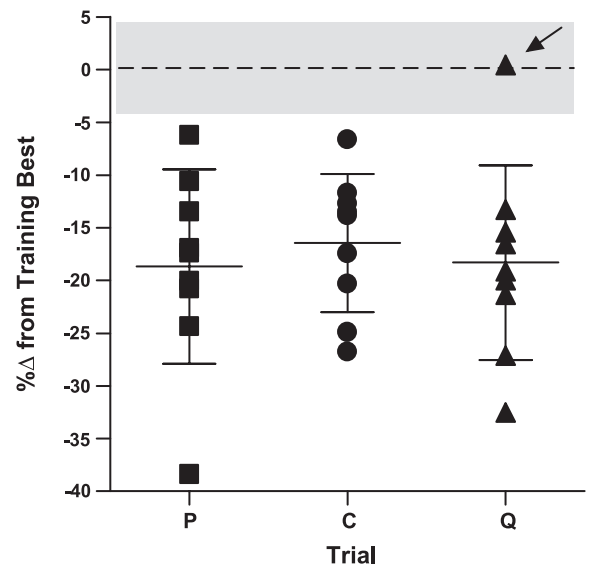

Fig. 2. Differences in pacing strategies between actual and normalized time blocks of work recorded at 3-min intervals over 15-min. Main effect of time, *less than block 1, #less than block 5. Dashed line represents an even pace where zero is the average (normalized) work performed in a 3-min work block (15/5). All notations are significantly different (P < 0.05).

a much larger study sample size will always improve the precision of the population performance estimate, narrow the confidence limits, and offer more certainty when trying to extrapolate findings (32), the results reported herein seem plausible, given that small ergogenic effects might not emerge under conditions that themselves substantially impair performance (heat) or increase performance variability (heat, treatment uncertainty).

In conclusion, the principle finding of this study was that acute supplementation with caffeine or quercetin did not improve endurance exercise performance in the heat. We also report a modest thermogenic effect of caffeine, but not quercetin, during exercise in the heat. Critical to accepting these conclusions is the recognition that *1*) blood concentrations of caffeine and quercetin were consistent with an ergogenic mechanism of adenosine receptor antagonism, *2*) potential study confounders for the heat were controlled, and *3*) methods and procedures deemed desirable for dietary supplement performance research (29, 32, 42) were in place.

Perspectives in Practice

Few ergogenic aids are recognized as legitimate. While the circumstantial efficacy for these are usually well characterized (e.g., endurance, speed, power), environmental considerations (heat, cold, altitude) are infrequently considered. Our approach, similar to one we have used before (8), was to study a well-recognized ergogenic aid (caffeine) and a novel supplement with similar potential actions (quercetin), under environmental heat-stress conditions. Like others (10, 21, 36), our data suggest that unique environmental circumstances may negate the efficacy of otherwise well-established nutritional ergogenic aids. This finding has clear importance for integrated physiology research, athletics, and military applications.

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